CLAIMS

1. A method for optimizing multiplex PCR primers, which method comprises:

a) providing a plurality of 5' and 3' specific primers, each of said specific primers comprising a specific sequence complementary to its target sequence to be amplified and a common sequence;

b) providing a 5' and a 3' universal primer, said 5' universal primer being complementary to said common sequence of said 5' specific primers and said 3' universal primer being complementary to said common sequence of said 3' specific primers;

c) conducting a plurality of multiplex PCRs on a plurality of target sequences in the presence of said plurality of 5' and 3' specific primers and said 5' and 3' universal primers, wherein in each of said PCRs, the concentration of said 5' and 3' universal primers equals to or is higher than the concentration of said 5' and 3' specific primers, respectively, and the concentrations of said 5' and 3' specific primers in different PCRs are different, respectively; and

d) assessing PCR products of said different PCRs and identifying a PCR wherein said target sequences are comparably amplified to identify optimized multiplex PCR primers for amplifying said target sequences in multiplex PCRs.

2. The method of claim 1, wherein at least one of the specific primers further comprises, at its 5' end, a sequence complementary to a sequence at its 3' end so that under suitable conditions, a hairpin structure is formed within the specific primer.

3. The method of claim 1, wherein the 5' and 3' specific primers and the 5' and a 3' universal primers are present in the multiplex PCRs simultaneously.

4. The method of claim 1, wherein the 5' and 3' universal primers are added into the multiplex PCRs after about 1-15 rounds of amplification using the 5' and 3' specific primers.

5. The method of claim 1, wherein the ratio between the 5' and 3' universal primers and the 5' and 3' specific primers is from about 1 to about 500.

6. The method of claim 1, wherein the concentration of the 5' and 3' universal primers is from about 0.01 μM to about 10 μM.

7. The method of claim 1, wherein the concentration of the 5' and 3' specific primers is from about 0.01 μM to about 1 μM.

8. The method of claim 1, wherein the GC content of the universal primers and/or the specific primers is from about 30% to about 80%.

9. The method of claim 8, wherein the GC content of the universal primers and/or the specific primers is from about 40% to about 60%.

10. The method of claim 1, wherein the difference of the GC contents of the specific sequence of the specific primers is within about 20%.

11. The method of claim 1, wherein the Tm of the specific sequence of the specific primers is from about 30°C to about 80°C and wherein the Tm is determined by the nearest neighbor method.

12. The method of claim 1, wherein the Tm of the specific sequence of the specific primers is from about 40°C to about 60°C.

13. The method of claim 1, wherein the difference of the Tm of the specific sequence of the specific primers is within about 20°C.

14. The method of claim 1, wherein the length of the universal primers and/or the specific primers is from about 10 nucleotides (nt) to about 40 nt.

15. The method of claim 14, wherein the length of the universal primers and/or the specific primers is from about 18 nt to about 25 nt.

16. The method of claim 1, wherein the difference of the universal primers and/or the specific primers is within about 10 nt.

17. The method of claim 1, wherein the PCR products are assessed via agarose gel electrophoresis.

18. The method of claim 17, wherein the difference of the length of the PCR products is more than about 30 base pairs (bp).

19. The method of claim 18, wherein the difference of the length of the PCR products is from about 30 bp to about 50 bp.

20. The method of claim 1, wherein the PCR products are assessed via polyacrylamide gel electrophoresis and capillary electrophoresis.

21. The method of claim 1, further comprising conducting multiplex PCR primers to amplify the target sequences using the identified optimized multiplex PCR primers.

22. The method of claim 1, wherein the target sequences are of viral, bacterial, fungal, plant, animal or human origin.

23. The method of claim 1, wherein the target sequences are derived from a virus that causes or is associated with the severe acute respiratory syndrome (SARS-CoV).

24. The method of claim 1, which is used to optimize primers for multiplex one-step RT-PCR.

25. The method of claim 1, which is used to optimize primers for multiplex nested PCR.

26. The method of claim 25, wherein in the multiplex nested PCR, both the first round and second round of amplification are conventional multiplex PCR.

27. The method of claim 25, wherein in the multiplex nested PCR, the first round of amplification is a multiplex one-step RT-PCR and the second round of amplification is a conventional multiplex PCR.

28. A composition for optimizing multiplex PCR primers, which composition comprises:

a) a plurality of different concentrations of a plurality of 5' and 3' specific primers, each of said specific primers comprising a specific sequence complementary to its target sequence to be amplified and a common sequence; and

b) a concentration of a 5' and a 3' universal primer, said 5' universal primer being complementary to said common sequence of said 5' specific primers and said 3' universal primer being complementary to said common sequence of said 3' specific primers,

wherein the concentration of said 5' and 3' universal primer equals to or is higher than any of the concentrations of said 5' and 3' specific primers, respectively.

29. A kit for optimizing multiplex PCR primers, which kit comprises:

a) a plurality of 5' and 3' specific primers, each of said specific primers comprising a specific sequence complementary to its target sequence to be amplified and a common sequence;

b) a 5' and a 3' universal primer, said 5' universal primer being complementary to said common sequence of said 5' specific primers and said 3' universal primer being complementary to said common sequence of said 3' specific primers;

c) means for conducting a plurality of multiplex PCRs on a plurality of target sequences in the presence of said plurality of 5' and 3' specific primers and said 5' and 3'

universal primers, wherein in each of said PCRs, the concentration of said 5' and 3' universal primers equals to or is higher than the concentration of said 5' and 3' specific primers, respectively, and the concentrations of said 5' and 3' specific primers in different PCRs are different, respectively; and

d) means for assessing PCR products in said different PCRs and identifying a PCR wherein said target sequences are comparably amplified to identify optimized multiplex PCR primers for amplifying said target sequences in multiplex PCRs.